Ribulose -1,5-bisphosphate carboxylase/ oxygenase, E.C.4.1.1.39 (RUBISCO) was discovered by Wildman & Bonner (1947). It is the most abundant enzyme in nature and it initiates as bifunctional enzyme for Photosynthetic reduction of CO₂ in the first step of the Photorespiratory pathway (Miziorko & Lorimer, 1983). RUBISCO is by far the best studied plant enzyme and its properties have been extensively reviewed (Andrews and Lorimer, 1987; Gutteridge, 1990). The ratio between the Carboxylase and Oxygenase reactions determines the efficiency of CO₂ fixation in determining plant productivity and genetic manipulation (Ellis and Gatenby, 1984).

The enzyme RUBISCO is composed of two subunits, one is coded for nuclear genetic material and the other for chloroplastic DNA. It has a bilayered structure each layer containing four large and four small subunits capped at both ends by small subunits of adjacent pairs. Despite the significant sequence conservation between large subunit (LSU) from various sources, the holozyme differs appreciably in their Carboxylation / Oxygenation ratios (Andrews & Lorimer, 1987), raising the possibility that the CO₂ / O₂ specificity of the holozyme is conferred by the small subunit (SSU). However, SSU has been shown to induce active site conformational changes. Experimental data indicate that the ratio of carboxylation to oxygenation is determined by the LSU alone (Andrews & Lorimer, 1985; Incharoensakdi et al., 1986). In a variety of plants the holozyme has a molecular weight of 530 - 560 KDa and it consists of eight large subunits (LSU), MW 53 - 55 KDa, each and eight small subunits (SSU), MW 12 - 15 KDa each depending on the species analysed (Leible et al., 1982; Miziorko & Lorimer, 1983; Duday et al., 1986).
The enzyme catalysed the Carboxylation of 1340 moles of ribulose bisphosphate per minute per mole of enzyme or the formation of 2680 moles of 3-phosphoglycerate per minute per mole under standard assay conditions. Ribulose bisphosphate itself became inhibitory at concentration exceeding 0.7 mM. Orthophosphate and ammonium sulphate were competitive inhibitors with respect to ribulose bisphosphate. Some effect on the photosynthesis could be mediated by RUBP regeneration and by RUBP carboxylase activation. The major biochemical process in the photosynthesis is considered to be the fixation of CO₂ by RUBP carboxylase/oxygenase and its further reduction by the Calvin cycle to form carbohydrates (Lorimer & Andrews, 1981). The biochemical processes are affected by temperature, carbon dioxide concentration and light intensity (Enoch et al., 1986). Temperature affects mainly Michallis constant and maximum velocity of the enzymic reactions. The temperature optimum for net assimilation rate in most C₃ plants is between 20° to 30° C under normal environmental conditions (Loirmer & Andrews, 1981). Since RUBPCase fixes CO₂ & O₂ competitively, increasing ambient CO₂ concentration results in increased gross photosynthesis due to advantage of CO₂ over O₂ in their competitive uptake. Reduction of O₂ uptake results in suppressed photorespiration and consequently CO₂ evolution resulting in increase in net assimilation.

It is found that RUBP carboxylase can alter genetic expression in tall fescue (Festuca arundinaceae Shreb). In oppose to a mean rate 32 to 41 mg CO₂ / dm² hour for 10 hexaploid genotypes the rate found here is 32 to 41 mg CO₂ / dm² hr. This decaploid genotype exhibits a ribulose-1,5-bisphosphate carboxylase specific activity 1.3 to 2.0 fold higher than typical tall fescue genotypes. Specific activities of photorespiratory enzymes & nitrate reductase
enzyme were lower in the decaploid than the hexaploid genotypes. Results suggest that genetic expression of RUBP carboxylase activity may have been altered to increase the net photosynthesis rate in the decaploid genotype (Randall et al., 1977). The variation between the enzymes from different organisms in the relative specificity for CO₂ and O₂ suggests that this varies according to the structure of the protein. Genes for several forms of the enzyme have been isolated and expressed in bacteria. These genes are now being manipulated in vitro in attempts to alter the relative rates of the carboxylase and oxygenase activities. Works has also carried out on the factors which may control the enzyme activity within the plant chloroplast.

An essential prerequisite for genetic manipulation of RUBISCO is the isolation of the structural genes and their expression in a suitable host, to produce sufficient quantities of enzyme such that the properties of mutant forms of the enzyme can be studied. The simplest form of RUBISCO is found in a photosynthetic bacterium, Rhodospirillum rubrum. This enzyme is composed of two identical subunits denoted as L₂. The gene coding for the protein was obtained from a clone library and inserted into plasmid PBR322, with part of the lacZ gene of the bacteriophage M₁₃mp7 included to increase expression (Somerville & Somerville, 1984). The resulting recombinant plasmid, Escherichia coli, where it produced relatively high levels of active enzyme. This plasmid has been used as the basis for site specific mutagenesis to modify the structure of the carboxylase, in order that the centrifugation of the specific amino acid residues to the kinetic parameters can be studied.

Using a combination of site directed mutagenesis and chemical modification, a 5-fold lowering of the substrate specificity of L₂ RUBISCO from
Rhodospirillum rubrum has been achieved (Smith et al., 1990). In addition, the roles of individual amino acid residues within the catalytic domain of the LSU of Rhodospirillum rubrum has been investigated using site directed mutagenesis (Murai et al., 1990).

This approach is dependent largely on the ability to express the mutagenised proteins in Escherichia coli and for this reason it has been successful with prokaryotic enzymes. The synthesis and the assembly of the higher plant enzyme is very complex, requiring additional proteins for assembly and activation, and it is not yet possible to produce an active $L_8S_8$ protein from higher plants in Escherichia coli (Christine et al., 1991).

The 3D structure of the RUBISCO enzyme has now been resolved (Anderson et al., 1989; Knight et al., 1989). The $L_8$ core of the enzyme is essentially a tetramer of interlocking dimers capped at both ends by small subunits, each of which is in contact with two large subunits of adjacent pairs. The LSU has two domains; the main structural component, an alpha/ beta barrel, is at the carboxyl terminus and this region interacts with the amino terminal domain of the adjacent LSU forming the active site between the large subunit dimer (Anderson et al., 1989). The SSU is essential for full catalytic activity of higher plant RUBISCO, although the mechanism of action is incompletely understood (Andrews, 1988).

The genes encoding the SSU and LSU were the first chloroplast (Bedbrook et al., 1979) and plant nuclear genes (Bedbrook et al., 1980) to be sequenced. Since that time a large number of both SSU (at least 33 from more than 10 species) and LSU genes have been sequenced. The sequences of the LSU is highly conserved, indeed the residues which bind the substrate are invariant in all RUBISCO studied to date, and it has been used extensively in phylogenetic
and evolutionary studies (Ritland & Clegg, 1987). This is not the case with SSU, where up to 20% divergence is possible (Manzara and Gruissman, 1988; Denn et al., 1989).

No Calvin cycle intermediate has yet been vigorously proved to have a differential effect of the Carboxylase / Oxygenase activities of the enzyme, although replacement of Mg$^{2+}$ by other divalent ions such as Mn$^{2+}$ or Co$^{2+}$ has been reported to produce different ratios of carboxylase / oxygenase activity (Christeller & Laing, 1979; Robinson et al., 1979).

Jordan and Ogren (1981, 1983) determined the kinetic parameters for RUBP Carboxylase isolated from organisms ranging from photosynthetic bacteria to higher plants and observed that Michaelis constant for CO$_2$ decreased as the evolutionary scale is climbed, and was less marked with a corresponding increase in the substrate specificity factor.

Efforts made to regulate photorepiration involve (i) blocking of the photorespiratory steps by chemical and genetic methods (ii) differential regulation of RUBP carboxylase / oxygenase activity by chemical as well as environmental factors and (iii) directly by altering the RUBISCO gene in vitro chemical like fluoride and ribulose-5-phosphate used inhibited phosphoglycolate phosphatase enzyme activity but failed to enhance net photosynthesis. Except for Manganese compounds that knock out oxygenase activity were found to affect carboxylase activity. With the increase in temperature RUBISCO affinity for CO$_2$ decreases but affinity of the enzyme for oxygen remains unaffected. Attempts are under way to increase the copy number of small subunit genes to see whether increased accumulation of RUBP carboxylase could increase
carboxylation (Lea & Stewart, 1984).

Menon (1987) observed increase in photosynthetic efficiency of crop plants together with increase in leaf area and its chlorophyll and carotenoid contents, control of stomatal opening and retardation of photorespiration through application of Mixtalol. Mixtalol also enhanced RUBPcarboxylase activity in C3 plants and PEPcarboxylase activity in C4 plants; bio-mass and crop yield improved by 20-50%. That the increased carboxylation can lead to increased crop yield was evident when CO2 concentration was increased five fold in open topped field enclosure in the period from anthesis to senescence (Hardy et al., 1978). Increases of crop yield of 50-100% were obtained for grain legumes, Soybean showing the maximum increase. Smaller increases of 10-50% were found for cereals supplied with CO2 prior to anthesis. These increased yields were attributed to a number of factors, the most important being a decrease in photorespiration and an increase in carboxylation caused by the elevated ratio of CO2 to O2. It was also reported that these encouraging results did not prove that photorespiration could be eliminated without harm to the plant but more realistic view was that photorespiration could be reduced under certain conditions. Lorimer and Andrews (1987) found that hybridisation between *synochococcus* large subunit and spinach small subunit increased the carboxylation rates than their parents. The inorganic phosphate (Pi) is reported to be a potent stimulator of the activity of RUBISCO (Brook et al., 1988).

Campbell et al. (1988) observed heavier Soybean pods at elevated CO2 concentration. However, RUBISCO activity in Soybean was not regulated by CO2 concentration within the range used.
Although, considerable work has already been done on photosynthesis in tea plants until recently no attempt has been made to study the activity of Ribulose-1,5-bisphosphate carboxylase / oxygenase in relation to photosynthetic capacity. Recently Aoki (1990) has tried to understand the cause of winter cold induced depression of photosynthesis in tea leaves. The overwintering leaves were harvested from the mature tea plants and changes in their photosynthetic capacity were measured in autumn and winter. Though photosynthesis and the light saturated Hill activity of the overwintering leaves corresponded to the changes in the photosynthetic rate, the soluble protein and the RUBPCase activity of the leaves appeared to have no correlation.

The pH optimum for the ribulose-1,5-bisphosphate carboxylase catalysed reaction in Spinach leaves was about 7.9. The Km values determined for ribulose-1,5-bisphosphate, Mg$^{++}$, total CO$_2$ (CO$_2$ + HCO$_3^-$) at pH 7.9 were 0.12 mM, 1.1 mM and 2.2 mM respectively (Wishnick et al., 1969).

Studies conducted in wheat, maize, tobacco, spinach and pea showed no difference in mass of the large subunit, polypeptides of the higher plant carboxylases (Parry et al., 1987). The small subunit polypeptides from spinach, pea and tobacco carboxylase had similar molecular weight of about 14,000. It is higher than that of small subunit of maize and lower than that of wheat.

The electrophoretic separation and subsequent studies of RUBISCO showed 25 distinguishable peaks in spongy parenchyma cells of *Vicia faba* (Tarezynski et al., 1988). The largest of this was RLSmp (migratory position of large subunit).

Though Mg$^{++}$ is needed for activation of the enzyme, it is not directly involved in the carboxylation reaction (Machler et al., 1979). Low phosphorous treatment did not decrease the total activities of ribulose-1,5-bisphosphate
carboxylase and the ribulose-5-phosphate kinase. Low phosphorous treatment affected photosynthetic rate through an effect on RUBP regeneration rather than through RUBPcase activity and that the changes in Calvin cycle enzymes with low phosphorous resulted in an increased flow of carbon to starch (Rao & Terry, 1989).

Isoelectric focusing (IEF) of RUBISCO gives information on both the chloroplast and nuclear genome. IEF of the enzyme in presence of 8M urea resolved the LSU as well as the SSU into polypeptides due to differences in isoelectric points (PI). LSU are encoded by nuclear genome as a small multigene family. Therefore, IEF of RUBISCO gives information on both, the chloroplast and the nuclear genome (Mummenhoff & Hurka, 1990).

IEF patterns of the LS are characterised by one dominant LS band (LS major band) and one or two LS minor bands of lower staining intensity. Altogether two LS major bands (LS I, LS II) and nine SS bands (SSI - SS9) forming seven different SS patterns, were displayed by the 14 Australian Lepidium species (Mummenhoff et al., 1992).

In C3 plants, the activity of RUBISCO is postulated to be regulated either by reversible carbamylation of a Lys residue in the catalytic site, enabling catalysis, or by the binding of inhibitors such as CA1P to carbamylated catalytic sites disabling catalysis (Seemann et al., 1990; Portis, 1992). Regulation of RUBISCO activity by reversible carbamylation occurs in response to changes in light intensity as well as the concentration of CO2 and O2, whereas inhibition of RUBISCO activity by CA1P occurs only in response to varying PPFD (Sharky et al., 1986; Sage et al., 1990; Seemann et al., 1990). At physiological levels of CO2 in C3 plants (5-10 μM), full carbamylation of RUBISCO can occur if CO2
levels are increased well above ambient (above 100 µM) in the absence of RUBP (Andrews & Lorimer, 1987).

Subjecting RUBISCO (holoenzyme, LSU and SSU) to IEF in 8 M urea resulted in dissociation of their constituent polypeptides and further separation of the individual polypeptides due to differences in isoelectric points (Mummenhoff & Hurka, 1990).

The photosynthetic capacity of leaves is closely related to their nitrogen content. As leaf nitrogen content increases, the photosynthetic rate at any partial pressure of CO₂ is enhanced (Makino et al., 1992). According to the photosynthetic model of Farquhar and Von Caemmerer (1982), the photosynthetic rate at low CO₂ partial pressure is limited by RUBISCO capacity, whereas the rate at high CO₂ is limited by electron transport capacity. In addition, photosynthesis under saturating CO₂ conditions can also be limited by the capacity of starch and Suc synthesis to regenerate Pi for photophosphorylation. Therefore, the increase in photosynthesis at all CO₂ partial pressures suggests that the capacity of each limiting process increases with increasing leaf nitrogen content. The ratio of RUBISCO to electron transport activities increases with increasing leaf nitrogen in many C₃ species (Makino et al., 1992). This relative increase in RUBISCO is considered to be required to maintain the balance between the in vivo capacities of RUBISCO and electron transport because of the presence of a CO₂ transfer resistance between intercellular air spaces and the carboxylation sites (Evans & Terashima, 1988).

Sage et al. (1990a), working with Chenopodium album, found that the CO₂ saturation point of photosynthesis decreases with increasing leaf nitrogen. Based on the assumption that photosynthesis at the CO₂ saturation point can be
limited by the capacity of starch and Suc synthesis, Sage et al. (1990b) have argued that the increase in the capacity of starch and Suc synthesis with increasing nitrogen is not as great as the increase in the capacity of RUBISCO and electron transport. However, none of the underlying biochemical reactions for the decline in the CO₂ saturation point has been reported. In addition, it is largely unknown how leaf nitrogen affects the capacity of starch and Suc synthesis.

The increase in the ratio of RUBISCO to total leaf nitrogen with supply is frequently found in many C₃ species (Makino et al., 1992). This relative increase in RUBISCO content is considered to be required because the partial pressure of CO₂ at the carboxylation site is reduced with increasing RUBISCO content by a CO₂ transfer resistance (Evans and Tarashima, 1988). The presence of a significant CO₂ transfer resistance between the intercellular air spaces and the carboxylation sites was originally pointed out by Evans (1983) because of a curvilinear relationship between CO₂ limited photosynthesis and the in vitro RUBISCO activity. In addition, the presence of this resistance has been confirmed by concurrent measurements of gas exchange and ¹³C discrimination during CO₂ uptake (Evans et al., 1986; Von Caemmerer & Evans, 1991) and by the analysis of the CO₂ sensitivity of photosynthesis using gas exchange and chl fluorecence measurements (Hardey et al., 1992; Loreto et al., 1992; Makino et al., 1994).

The above status and the literature cited in Chapter II prompt the present investigation to study the properties of RUBISCO in tea and its impact on the screening of superior cultivars in respect of crop yield. Further, literature are reviewed in the subsequent chapters also where and whenever they are felt to be of relevance.